Intraepidermal Psoriatic Cytokine Network Involves Gamma Interferon, Transforming Growth Factor-Alpha, and Their Cell Surface Receptors: Dysregulation Rather than Deficiency

To the Editor:

We read with interest the report by Scheynius et al [1] documenting the lack of gamma interferon (IFN-γ) receptors in the upper epidermal layers of psoriatic lesions. Their direct in vivo immunohistochemical documentation of this aberrancy regarding psoriatic keratinocyte's (KC) IFN-γ receptor expression places yet another piece of this complex cutaneous puzzle into place. Indeed, the cytokine network operative in psoriatic lesions is being rapidly dissected at the molecular level [2]. Their observation is in line with the notion that the KC hyperproliferation and lack of diffuse HLA-DR expression, despite the presence of IFN-γ (a potent cultured KC growth inhibitor and inducer of HLA-DR) being produced in the epidermal compartment [3], may reflect loss of growth inhibition secondary to dysregulated or deficient expression of IFN-γ receptors [2]. We would like to supplement their report with the following observations from our laboratory, which are relevant to this topic, and reveal that further investigation into the reciprocal interactions between IFN-γ, transforming growth factor-alpha (TGF-α), and their cell surface receptors are indicated.

Our approach to understanding the pathophysiology of psoriasis (and perhaps its genetically transmissible etiology), has been to isolate and propagate cultured KC derived from psoriatic lesions using a serum-free low-calcium medium (KGM, Clonetics Corp; San Diego, CA) to compare/contrast their behavior after multiple passages (and hence separated from their immediate microenvironmental influences) with KC from normal skin or other T-cell–mediated diseases. We believe that "mutant" KC are in fact capable of being perpetuated in vitro because the KC derived from psoriatic lesions are less responsive to the antiproliferative and immunomodulatory effects of IFN-γ [4,5], whereas cultured cells from normal skin or lesion of atopic dermatitis and cutaneous T-cell lymphoma do not have these characteristics. In subsequent unpublished studies we asked whether the psoriatic KC expressed any high-affinity IFN-γ receptors at all, i.e., was the altered response due to a deficiency or dysregulation of IFN-γ receptors? By ^{32}P–IFN-γ ligand binding and Scatchard analysis of multipassaged psoriatic KC as previously described [6] we determined that for three different patients, their KC express less than 50% (all values represent mean of triplicate wells) the number of high-affinity receptors (5800, 9000, and 6100 sites/cell) compared to normal KC (22,000 sites/cell), without any significant alteration in the binding constant ($K_d = 0.22$ nM). Northern blot analysis of IFN-γ receptors revealed that psoriatic KC could indeed produce an appropriate-size mRNA coding for the IFN-γ receptor. For these experiments, 10 μg of total cellular RNA was placed in each lane and separated according to molecular weight on 1% agarose gels containing 8% formaldehyde and electroblotted onto a nylon membrane as previously described [5], the blots were hybridized with a ^{32}P-labeled cDNA insert obtained from Dr. S. Pestka, pHuIFN-γR8 [7]. After washing and autoradiography, a single approximately 2.3-Kb transcript was identified (Fig 1). When the normal and psoriatic KC were treated with various cytokines (i.e., IFN-γ, TNF-α) or phorbol ester (alone and in combination), there were changes in the level of expression of the IFN-γ receptor mRNA but, in this context, the most important point is that the psoriatic KC did produce such an mRNA species that was comparable between the normal and psoriatic cells. These treatment regimens were selected based on our previous experience in modulating various KC-derived adhesion molecule and chemotaxin mRNA [5]. Moreover, when we have performed a karyotypic analysis of three different multipassaged psoriatic KC that had an aberrant phenotype, (↑, TGF-α production; ↓, IFN-γ receptors and responsiveness), no obvious abnormality (i.e., deletion) in the long arm of chromosome 6 (region coding for IFN-γ receptor) was observed (Fig 2). In these psoriatic cultures, two cell populations of KC could be identified morphologically and by chromosomal analysis. One population was diploid (Fig 2*A*) without any abnormality, but the other population was near-tetraploid, and contained a marker chromosome (Fig 2*B*). Interestingly, the same marker chromosome was seen in three different patients, which resembled chromosome 16. Thus, the simplistic suggestion [2] that the psoriatic gene may involve deletion of the IFN-γ receptor DNA coding sequence is apparently incorrect. To further investigate whether the diminished level of IFN-γ receptors expressed on the surface of psoriatic KC was due to a dysregulatory event involving other cytokines, we returned to our earlier observation that psoriatic KC overproduced TGF-α (approximately twofold) compared to normal KC [8].

We asked whether increased TGF-α levels could influence IFN-γ receptors on normal KC as a mechanism to explain our observations involving psoriatic KC. Indeed, we observed that, by adding either epidermal growth factor (EGF) or recombinant TGF-α to cultured KC maintained in a basal medium devoid of any growth supplements, both these mitogens markedly reduced high-affinity KC IFN-γ receptors and their immunologic responsiveness [6]. Thus, TGF-α can reduce IFN-γ receptors, which extended our earlier reports that IFN-γ could reduce EGF receptors [9], as well as increase TGF-α mRNA and protein production [7]. We believe this in vitro link between TGF-α and IFN-γ receptors is relevant to the in vivo report [1] because the enhanced expression of TGF-α protein and mRNA that has been documented by several laboratories [10–12] tends to be most prevalent particularly in suprabasal KC, which is exactly where the greatest reduction in IFN-γ receptors was noted.

In conclusion, there appears to exist cross-talk between the pro-

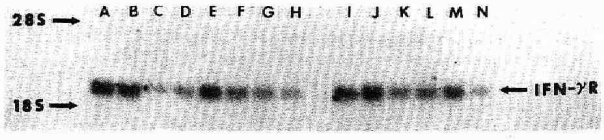

Figure 1. Northern blot hybridization of cultured KC with an IFN-γ receptor cDNA probe reveals a single 2.3-Kb transcript. *Lanes A-H,* normal KC, *lanes I–N,* psoriatic KC. Treatments. *Lanes A and I: KGM,* 24 h. *Lanes E and J: KGM,* 48 h. *Lanes B and K:* IFN-γ 100 U/ml, 24 h. *Lanes F and L:* IFN-γ 100 U/ml, 48 h. *Lane C:* 12-0-tetradecanoyl-phorbol-13-acetate (TPA) 5 nM, 24 h. *Lane G:* TPA, 5 nM, 48 h. *Lane D:* IFN-γ (100 U/ml) plus TPA (5 nM), 24 h. *Lane H:* IFN-γ (100 U/ml) plus TPA (5 nM), 48 h. *Lane M:* TNF-α (250 U/ml), 24 h. *Lane N:* IFN-γ (100 U/ml) plus TNF-α (250 U/ml), 48 h.

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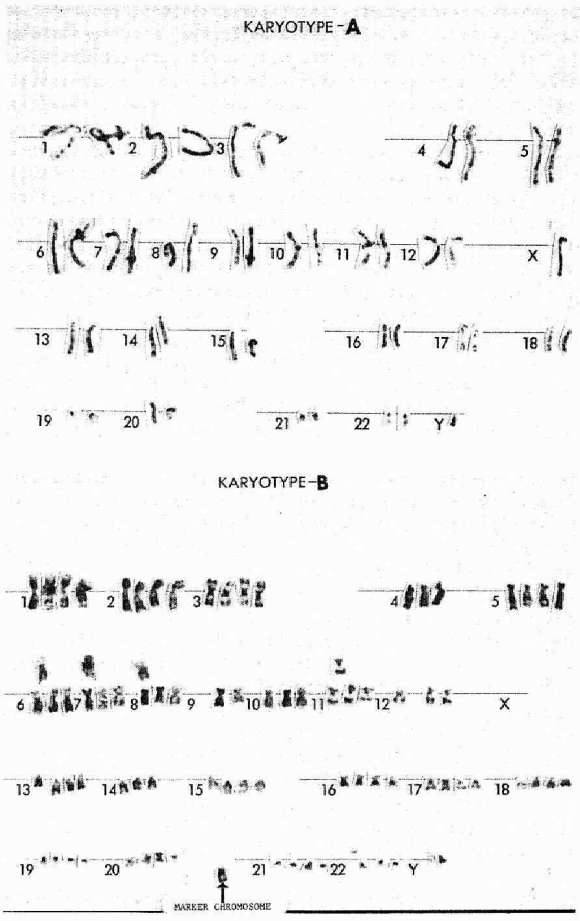

Figure 2. Karyotypic analysis of cultured multipassaged psoriatic KC that displayed an altered phenotype (kindly performed by Dr. Sue Sheldon, University of Michigan) revealed two cell populations *(A,B)*. Note that in both populations, the long arm of chromosome 6 is not deleted.

proliferative signaling pathways involving TGF-α/EGF receptors and the anti-proliferative pathway involving IFN-γ/IFN-γ receptors. Understanding these pathways in psoriatic lesions will not be easy, but it is becoming evident that, as we embark on this investigative journey, we can be re-assured that the pathway is relevant to this disease process, which manifests itself by cutaneous erythematous scaling plaques.

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